

DETAILED ACTION

Status of the Claims

Claims 1-25 are pending in the instant application; claims 4-6, 18, and 22, are withdrawn from consideration; claims 1-3, 7-17, 19-21 and 23-25, are the subject of the Office Action below.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-3, 7-17, 19-21 and 23-25, are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 12 are indefinite for reciting the phrase “the embedded polynucleotide” in step c) because there is insufficient antecedent basis for this limitation. The fact that there is a contacting in step b) does not clearly establish that the contacting is an “embedding” contacting. Correction is required.

Claim Rejections - 35 USC § 112

Claims 1-3, 7-17, 19-21 and 23-25, are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement for having new matter. The claims contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Claims 1 and 12 are rejected for adding the limitation “embedded” without adequate support in the specification. Neither the description nor the examples indicate that the polynucleotide is “embedded” in the matrix as claimed. There is neither literal support for the term, nor description that adequately suggests the breadth of the claimed language. Correction is required.

Claim Rejections - 35 USC § 102 - Withdrawn

The rejection of claims 1-3, 7-17, 19-21 and 23-25, under 35 U.S.C. 102(b) as being anticipated by Guerlavais *et al.*, *Analytical and Bioanalytical Chemistry*, 374:57-63 (2002), is withdrawn for the reasons argued by Applicants.

The rejection of claims 1-3, 7-17, 19-21 and 23-25, under 35 U.S.C. § 102(b) as being anticipated by Koster *et al.*, U.S. Patent No. 6,074,823, issued on June 1, 2000, is withdrawn in view of Applicants' amendments to the claims wherein the matrix is an "embedded matrix."

Applicants' arguments regarding "un-degraded" oligonucleotides are not found unpersuasive because this limitation is not part of the claims.

Claim Rejections - 35 USC § 103 – Necessitated by Amendment

The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. § 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-3, 7-17, 19-21 and 23-25, are rejected under 35 U.S.C. § 103(a) as being unpatentable over Koster *et al.*, U.S. Patent No. 6,074,823, issued on June 1, 2000 (Koster #1), in view of Koster *et al.*, U.S. Patent No. 6,043,031, issued on March 28, 2000 (Koster #2).

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Claim 1 is directed to a method of analyzing a polynucleotide using matrix assisted laser desorption/ionization mass spectrometry, the method comprising a) obtaining the polynucleotide bound to a substrate via a linker moiety, the linker moiety comprising a triaryl methyl linker group wherein the polynucleotide is bound to a substrate via the triaryl methyl linker group; b) contacting the polynucleotide bound to the substrate with a matrix material; c) inserting the substrate and the matrix embedded polynucleotide on the substrate into the source and d) analyzing the polynucleotide by matrix assisted laser desorption/ionization mass spectrometry.

Koster #1 teaches a fast and highly accurate mass spectrometer based processes for directly sequencing a target nucleic acid (or fragments generated from the target nucleic acid), which by means of protection, specificity of enzymatic activity, or immobilization, derivatives or truncated sequences detected by mass spectrometry using MALDI-TOF. In Fig. 23, Koster #1 teaches that nucleic acid immobilization is carried out using covalent bifunctional trityl linkers, and hydrophobic trityl linkers in Fig. 24 and teaches carrying out the synthesis on the substrate (compare to claims 2, 3 and 21-23). Koster states:

“In general, when it is the released nucleotide (or ribonucleotide) which is mass-modified, the modification should take as few steps as possible and be relatively efficient. For example, reactions used in adding base protecting groups for oligonucleotide synthesis can also be used to modify the released nucleotide just prior to mass spectrometric analysis. For instance, the amino function of adenine, guanine or cytosine can be modified by acylation. The amino acyl function can be, by way of illustration, an acetyl, benzoyl, isobutyryl or anisoyl group. Benzoylchloride, in the presence of pyridine, can acylate the adenine amino group, as well as the deoxyribose (or ribose) hydroxyl groups. As the glycosidic linkage is more susceptible to hydrolysis, the sugar moiety can be selectively deacylated if the acyl reaction was not efficient at those sites (i.e. heterogeneity in molecular weight arising from incomplete acylation of the sugar). The sugar moiety itself can be the target of the mass-modifying chemistry. For example, the sugar moieties can be acylated, tritylated, monomethoxytritylated, etc. Other chemistries for mass-modifying the released nucleotides (or ribonucleotides) will be apparent to those skilled in the art.

In another embodiment, the linear, single-stranded DNA fragment can be anchored to a solid support. This can be achieved, for example, by covalent attachment to a functional group on the solid support, such as through a specific oligonucleotide sequence which involves a spacer of

sufficient length for the ligase to react and which is covalently attached via its 5' end to the support (FIG. 1). A splint oligonucleotide with a sequence complementary in part to the solid support-bound oligonucleotide and to the 5' end of the linearized single stranded vector DNA allows covalent attachment of the DNA to be sequenced to the solid support. After annealing, ligation (i.e. with T4 DNA ligase) covalently links the solid support-bound oligonucleotide and the DNA to be sequenced. The splint oligonucleotide can be subsequently removed by a temperature jump and/or NaOH treatment, or washed off the support using other standard procedures. The solid support with the linear DNA is transferred to the reactor (FIG. 9) and contacted with an exonuclease in solution. As illustrated, where the 3' end of the unknown DNA fragment is exposed (i.e. unprotected), a 3' exonuclease is employed. The released nucleotides, or modified nucleotides, if intermediately contacted with a modifying agent such as alkaline phosphatase, are identified by mass spectrometry as described above. Other linking groups are described herein, and still others will be apparent to those skilled in the art based on the embodiments described herein. For example, the immobilization can occur through a covalent bond, such as a disulfide linkage, leuvalinyl linkage, a peptide/oligo peptide bond, a pyrophosphate, a tritylether or tritylamino linkage, which can be cleaved in accordance with standard procedures (see e.g. Example 14 and FIG. 23). Immobilization can also be obtained by non-covalent bonds such as between biotin and streptavidin or hydrophobic interactions (see e.g. Example 15 and FIG. 24)."

Koster, col. 9, line 49 through col. 10, line 37.

As in claim 7, the above teaching meets the limitation of direct linking to the triaryl methyl linker group. As in claims 8 and 24, an ionizing source generates nucleotide ions that are measured by the mass spectrometer. As in claims 9 and 25, the sample is adapted for MALDI-MS (cols. 3 and 4; see also Example 15). The structure of the compound in Koster meets the limitations of claims 10-17 and 19-20 (see Koster, Figure 23 and description thereof).

Although Koster #1 teaches the use of a matrix, such as hydroxy picolinic acid, Koster #1 does not explicitly teach using an embedded or dried from of the matrix.

Koster #2 a fast and highly accurate mass spectrometer based processes for detecting a particular nucleic acid sequence in a biological sample (see Abstract). Koster teaches that in one embodiment, a nucleic acid molecule containing the nucleic acid sequence to be detected (i.e. the target) is initially immobilized to a solid support. Immobilization can be accomplished, for example, based on hybridization between a portion of the target nucleic acid molecule, which

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is distinct from the target detection site and a capture nucleic acid molecule, which has been previously immobilized to a solid support. Alternatively, immobilization can be accomplished by direct bonding of the target nucleic acid molecule and the solid support. Koster #2 teaches a preference for a spacer (e.g. a nucleic acid molecule) between the target nucleic acid molecule and the support. A detector nucleic acid molecule (e.g. an oligonucleotide or oligonucleotide mimetic), which is complementary to the target detection site can then be contacted with the target detection site and formation of a duplex, indicating the presence of the target detection site can be detected by mass spectrometry. In preferred embodiments, the target detection site is amplified prior to detection and the nucleic acid molecules are conditioned. In a further preferred embodiment, the target detection sequences are arranged in a format that allows multiple simultaneous detections (multiplexing), as well as parallel processing using oligonucleotide arrays ("DNA chips"). Koster #2 teaches drying the matrix solution on the sample so as to embed the sample within the matrix:

“A suspension of streptavidin-coated magnetic beads with the immobilized DNA was pipetted onto the sample holder, then immediately mixed with 0.5 ul matrix solution (0.7 M 3-hydroxypicolinic acid in 50% acetonitrile, 70 mM ammonium citrate). ***This mixture was dried at ambient temperature and introduced into the mass spectrometer.***”

Koster #2, col. 25, lines 38-43 (emphasis added).

One of ordinary skill in the art would have had a reasonable expectation of success in arriving at the invention as claimed because each of Koster #1 and Koster #2 are directed to the use of MALDI-MS for oligonucleotide sequence determination wherein the oligonucleotides are immobilized to a solid substrate via trityl linkers and treated with matrix. One of ordinary skill in the art would have recognized the advantages of using an “embedded” matrix system as taught by Koster #2 with the approach of Koster #1, such as the high throughput assay format on the array of Koster #2. Therefore, the invention was *prima facie* obvious as a whole.

Common Ownership of Claimed Invention Presumed

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the Examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any

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evidence to the contrary. Applicant is advised of the obligation under 37 CFR § 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the Examiner to consider the applicability of 35 U.S.C. § 103(c) and potential 35 U.S.C. §§ 102(e), (f) or (g) prior art under 35 U.S.C. § 103(a).

Conclusions

No claim is allowable.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

If Applicants should amend the claims, a complete and responsive reply will clearly identify where support can be found in the disclosure for each amendment. Applicants should point to the page and line numbers of the application corresponding to each amendment, and provide any statements that might help to identify support for the claimed invention (*e.g.*, if the amendment is not supported *in ipsius verbis*, clarification on the record may be helpful). Should Applicants present new claims, Applicants should clearly identify where support can be found in the disclosure.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Jeff Lundgren whose telephone number is 571-272-5541. The Examiner can normally be reached from 7:00 AM to 5:30 PM.

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If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, James (Doug) Schultz, can be reached on 571-272-0763. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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